Aluminum Profiles in the Cerebrospinal Fluid of Patients in the Spectrum of Alzheimer's Disease. Relation to Classical Biomarkers and Indicators of Oxidative Stress

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Abstract

Alzheimer´s disease (AD) is a complex neurodegenerative disease characterized by progressive destruction of mainly brain cortical areas related to cognitive and memory performances. Most identified forms of AD are idiopathic and the precise pathogenic mechanisms remain yet unknown. Neuropathological hallmarks of AD include amyloid plaques and neurofibrillary tangles whose development correlate with neuronal death. At present, there is evidence that factors other than aging and genetic prone to develop AD contribute as cofactors in neuropathological cascades. Aluminum is considered a neurotoxic metal for humans. Some previous studies have revealed the accumulation of aluminum in the cerebral parenchyma of *postmortem* brains of patients suffering AD. In this study, we show that aluminum is present in significant amounts in the cerebrospinal fluid (CSF) of control healthy individuals and that its concentration increases in individuals with AD. Aluminum in CSF is also elevated in patients with mild cognitive impairment (MCI), considered to be a prodromal stage of AD. Aluminum contents correlate with classical biomarkers of AD (particularly with phosphorylated tau and amyloid β) following complex association patterns which vary depending on the stage of the AD spectrum, and dismiss a direct relationship. Associated with increased aluminum in AD patients, indicators of oxidative stress, namely Iso-PG2 and MDA, are also increased in the CSF of AD and MCI individuals, which also correlate with aluminum concentration. We hypothesize that aluminum content in CSF is a significant factor which, in synergy with other variables, may favors the initiation and progression of AD.

Keywords: *Alzheimer's Disease; Cerebrospinal Fluid; Aluminum; Oxidative Stress; Mild Cognitive Impairment; Phospho-Tau*

Abbreviations

CSF: Cerebrospinal fluid, SMC: Subjective Memory Complaints, MCI: Mild Cognitive Impairment, AD: Alzheimer´s disease, MDA: Malonyl-dialdehyde

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly in developed countries, affecting approximately 68% of all individuals over the age of 65 years. AD is characterized by the accumulation of amyloid β deposits in the brain parenchyma known as senile plaques and by the presence of neurofibrillary tangles (NFT) formed by hyperphosphorylated tau.

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Although the initial molecular events causing AD are not known, in recent years it has become evident that environmental, nutritional and lifestyle factors may act as synergistic conditions for developing AD [1,2]. Dietary aluminum has been implicated in the pathogenesis of AD based on epidemiological studies [3,4] as well as the detection of Al in NFT and senile plaques [5,6]. Currently, apart from drinking water, aluminum is extensively used in the industry and is added to a vast number of daily products (from processed foods to medical preparations). This widespread use of Al makes human exposure practically unavoidable.

In vitro and *in vivo* studies have shown that Al exposure can induce several deleterious effects implicated in neurodegenerative disorders, including AD pathogenesis [7,8]. However, conflicting results have been reported, and the issue of a direct involvement of Al in AD pathogenesis ("The Aluminum hypothesis") is still controversial and remains unresolved.

Aim of the Study

With the aim of adding novel insights into the cause-effects relationship between Al and AD, in the present study we have explored, in human CSF from different groups of subjects within the AD spectrum, the coexistence and potential links between aluminum and main hallmarks of Alzheimer´s disease.

Materials and Methods

Patients

A cohort of 68 patients was recruited at the Hospital Universitario de Gran Canaria Dr. Negrín (Spain). All procedures complain the directives of the Ethics Committee of the Hospital according to the Helsinki Declaration (1983). Based on the results of a full cognitive assessment including neuropsychological tests (Mini Mental State Examination test and In-Out test [9], the analyses of classical cerebrospinal fluid biomarkers (amyloid β, total tau protein and phosphorylated tau), daily life activities and the assessment of Global Deterioration Scale (GDS), subjects were classified into four groups: 1) Cognitively normal as internal controls (CTRL, 15 cases, average age 72.2 years); 2) Subjective Memory Complaints (SMC, 9 cases, average age 69.77 years); 3) Mild Cognitive Impairment (MCI, 29 cases, average age 74.72 years) and 4) Alzheimer´s disease (AD, 15 cases, average age 72.93 years).

Cerebrospinal fluid (CSF) samples

CSF samples were obtained by lumbar puncture in polypropylene tubes following a standard procedure. Once collected, samples were centrifuged at 2000g for 20 minutes at 4ºC and frozen at -80ºC until analysis.

Aluminum analysis by ICP-MS-MS

CSF samples were thawed slowly at 4ºC before being diluted 1:20 in a solution of 5% HNO_3 with 1000 ppm of Rh as internal standard. Quality control samples were analyzed along with the collected samples. In the inductively-coupled plasma mass spectrometry analysis, a lyophilized serum control for trace elements (ClinChek® Controls, Recipe, Germany) was used. Aluminum concentration was determined by interpolating the absorbance value in a multitrace standard curve and expressed as μmol/ml CSF.

Determination of classical AD and oxidative stress biomarkers.

Levels of classical biomarkers, i.e. Amyloid $β_{1-42}$ (Aβ), total tau (t-tau) and phosphorylated tau_{p181} (p-tau), were determined in the CSF of patients from the four groups using appropriate ELISA-kits (INNOTEST®, Fujirebio, Ghent, Belgium).

Indicators of oxidative stress, malonyldialdehyde (MDA) and 8-Iso-PGF2α, were also analyzed in the whole cohort. MDA contents were determined in 0,010 mL CSF following the method described by Ohkawa., *et al*. [10]. This technique is based on the reaction of MDA with thiobarbituric acid (TBA) to generate an MDA-TBA adducts. MDA-TBA adduct is a chromogen with absorbance peak at λ = 532 nm and whose formation is directly proportional to the presence of MDA in the sample.

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Isoprostane 8-Iso-PGF2α was determined in 0,55 mL CSF aliquots using a commercial specific kit OxiSelectTM 8-iso-prostanglandin kit F2α (Cell Biolabs, UK) following the manufacturer's instructions.

Statistics

Comparisons of individual variables between groups were performed by one-way ANOVA followed by Tukey's or Games-Howell post-hoc tests were appropriate. Bivariate relationships were performed using nonlinear regression methods. Parameters describing each regression analyses are shown in each figure.

Results and Discussion

We show here that significant aluminum contents can be detected in the CSF of all groups (Figure 1a). Aluminum values were similar between CTRL and SMC groups, but significantly higher in the MCI and AD groups ($p < 0.05$). Our data agree with previous studies in humans which have demonstrated elevated levels of aluminum in postmortem brain from subjects suffering Alzheimer`s disease, associated to cognitive functions, in particular the frontal cortex, the entorhinal cortex, the amygdala and hippocampus [7]. According to our results elevated Al is already present in the CSF of MCI individuals at similar levels than in AD. It is also relevant to note the wide range of aluminum values observed in the MCI and AD groups (0.01 - 1.76 in MCI and 0.09 - 2.03 in AD), compared to CTRL+SMC (0.01 - 0.71), which suggest that the relation between aluminum and AD does not reflect a direct cause-effect relationship (see below).

We have also determined the contents of classical biomarkers. In a previous study from our group [9], we showed in these same patients that p-tau levels were increased in the MCI (prodromal AD) and AD groups, as compared to individuals with normal psychometric performance (our current CTRL group), while Amyloid β was significantly lower in AD (and to a lesser extent in MCI) than in controls or SMC. Here we have further analyzed the relationship between aluminum and classical biomarkers. The results in figure 1b show that p-tau is positively related to aluminum in CTRL+SMC groups, suggesting a direct relation between the two variables. However, such relationship does apply to neither MCI nor AD groups (Figure 1c). Rather, it can be observed that a negative exponential regression line best defines the relationship between the two variables. The interpretation of these findings is that although levels of Al, as well as those of p-tau, are higher in MCI and AD groups, aluminum itself does not trigger the phosphorylation of tau.

Regarding Aβ, a similar regression analyses reveals a trend to reduce Aβ levels as Al concentration increases (Figure 1d), but the regression outcomes are far from reaching statistical significance ($p > 0.1$, correlation coefficients below 0.3). This is compatible with the observation that Aβ levels are significantly lower in AD group than in CTRL or SMC groups. Again, as in the case of p-tau, the interpretation of these findings is that Al contents themselves do not explain variations in Aβ levels between groups. Overall, Al does not seem to directly modulate neuropathological hallmarks of Alzheimer's disease, but rather it participates in multiple neuropathological events occurring in AD by altering a number of important processes for brain functioning [6,8], most of which has been demonstrated to occur in AD brains. These effects include cell signaling, axonal transport, neurotransmitter synthesis/release/uptake, synaptic transmission, phosphorylation/dephosphorylation of proteins, protein aggregation/degradation, amongst other processes [6,8,11]. Aluminum also plays a role in brain neuroinflammation by increasing glial activation and inflammatory response [8,12]. Thus, we conclude that accumulation of Al in brain parenchyma exacerbates AD symptoms, by adding deleterious effects on nerve cell physiology, rather than by stimulating Aβ burden and NFT formation.

A potential link between Alzheimer´s disease and aluminum is oxidative stress. It is well known that Al is capable of promoting free radical generation, despite the fact that it is not a valence-labile metal and does not have a react with sulfhydryl radicals. It has been postulated that Al may act by catalyzing the redox activity of trace amounts of iron. In our current study we have assessed the possible association of Al levels and oxidant status of CSF by determining levels of lipid-derived oxidized by-products, namely MDA and isoprostane 8-Iso-PGF2α. The results in figure 2 reveal that levels of 8-Iso-PGF2α (but not MDA) are significantly higher in AD and MCI groups com-

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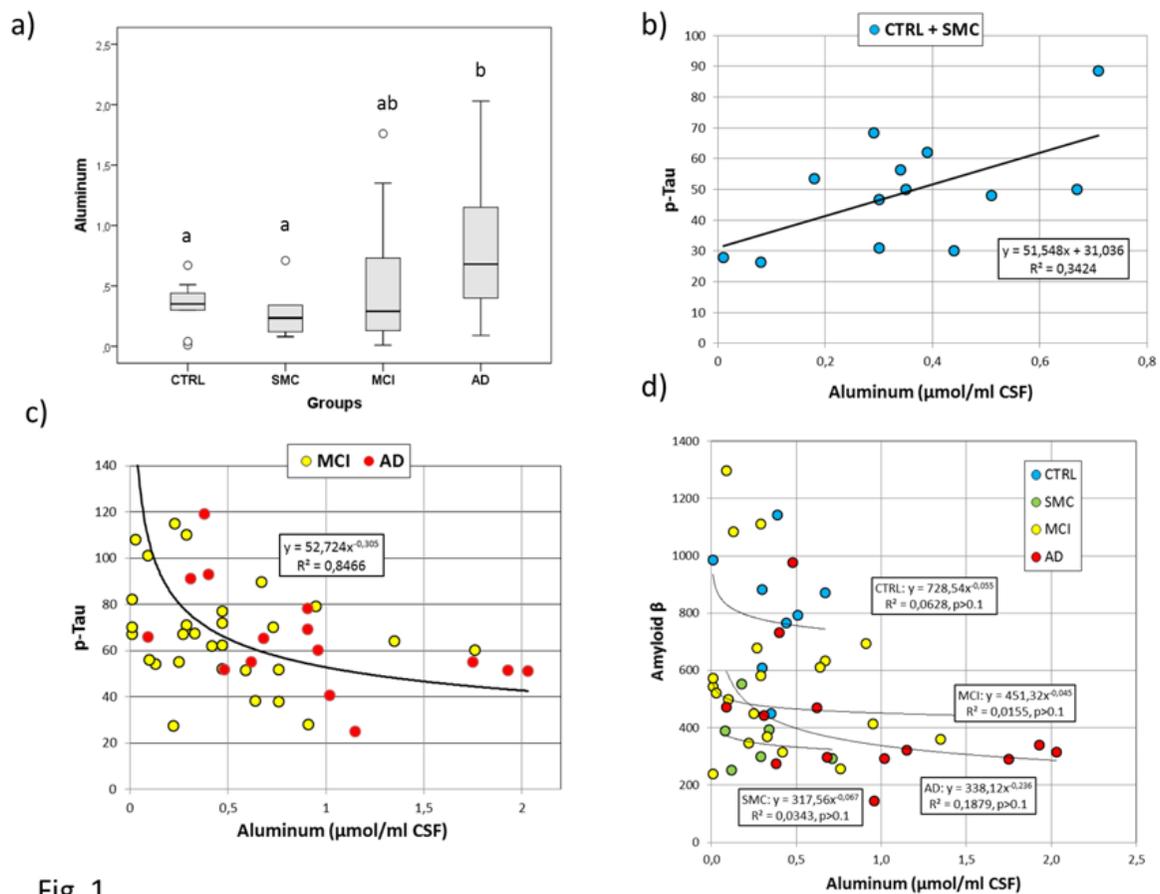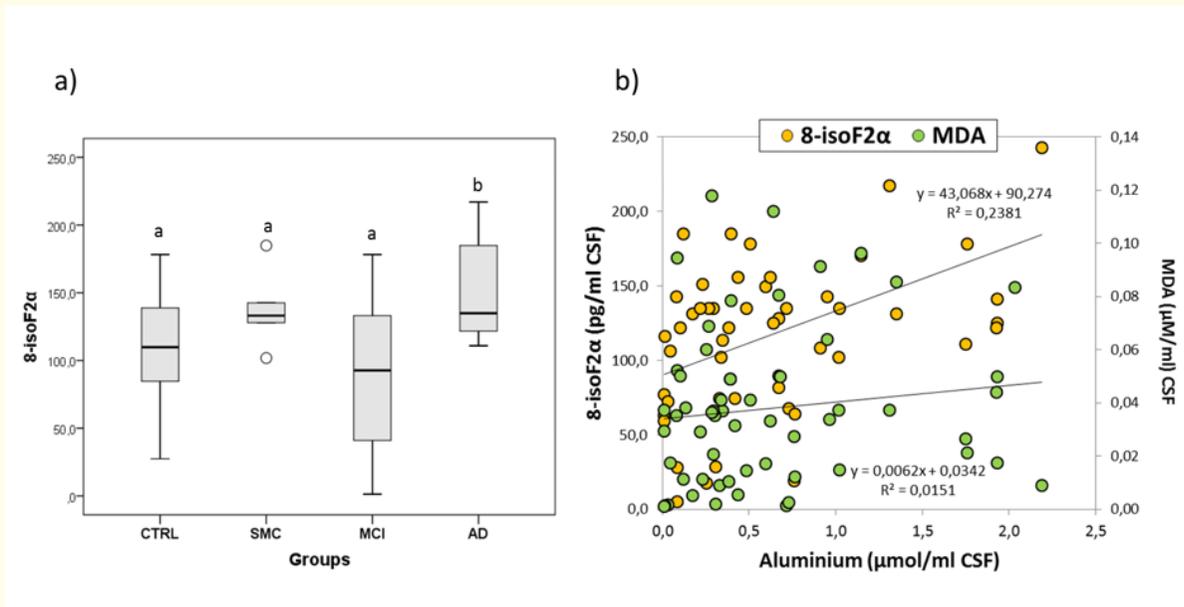
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Figure 1: *a) Box-plot of Al contents in the CSF of the different groups in the present study. Different letters indicate significant differences between groups at p < 0.05. Empty circles indicate potential outliers. b) Bivariate relationship between Al and p-tau in pooled CTRL and SMC groups. Lineal regression parameters are indicated. c) Bivariate relationship between Al and p-tau in MCI (yellow circles) and AD (red circles) groups. Lineal regression parameters are indicated. d) Bivariate relationship between Al and Amyloid β in CTRL (blue circles), SMC (green circles), MCI (yellow circles) and AD (red circles) groups. Lineal regression parameters are indicated. Note the negative exponential fitting of data in c) and d). R2: determination coefficient.*

pared to CTRL and SMC groups (Figure 2a), suggesting higher oxidant levels in prodromal (MCI) and established AD groups. The direct relationship between Al and oxidative biomarkers was demonstrated by regression analyses shown in figure 2b. A positive relationship between Al and 8-Iso-PGF2α was obtained, with higher values of Al in any group corresponding to higher lipoperoxide levels. These findings indicate that oxidative stress depends on the levels of Al in CSF rather than on belonging to a specific assigned group. This finding may explain why higher Al levels in AD patients associate with larger oxidative stress indicators in AD group, although not necessarily significantly increased with respect to the age-matched control group.

Citation: Mesa-Herrera., *et al.* "Aluminum Profiles in the Cerebrospinal Fluid of Patients in the Spectrum of Alzheimer's Disease. Relation to Classical Biomarkers and Indicators of Oxidative Stress". *EC Pharmacology and Toxicology* 9.2 (2021): 15-20.

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Figure 2: A) Box-plot representation of isoprostane 8-Iso-PGF2α contents in the CSF of the different groups in the present study. Different letters indicate significant differences between groups at p < 0.1. Empty circles indicate potential outliers. B) Bivariate relationship between Al and MDA (green circles) or 8-iso-F2a (orange circles) in the whole dataset. Lineal regression parameters are indicated. R^2: determination coefficient.

Conclusion

The in-depth analyses of the results presented here lead us to conclude that although Al contents are higher in the CSF of AD and MCI patients than in controls and asymptomatic groups, there is no direct association with paradigmatic biomarkers of AD. Instead it seems that Al contributes to AD neuropathology by triggering disruptive changes in nerve cell metabolism and physiology, including oxidative stress. Therefore Al appears to exacerbate AD symptoms in parallel with the ongoing AD pathological programme.

Acknowledgements

Conflict of Interest

Authors declare no financial interest or any conflict of interest.

Bibliography

1. Selkoe D. "Cell Biology of the Amyloid β-Protein Precursor and the Mechanism of Alzheimer's Disease". *Annual Review of Cell and Developmental Biology* 81.2 (1994): 373-403.

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2. Bettens K., *et al*. "Current Status on Alzheimer Disease Molecular Genetics: From Past, to Present, to Future". *Human Molecular Genetics* 19.1 (2010): 1-8.

3. McLachlan DRC., *et al*. "Risk for Neuropathologically Confirmed Alzheimer's Disease and Residual Aluminum in Municipal Drinking Water Employing Weighted Residential Histories". *Neurology* 46.2 (1996): 401-405.

4. Rondeau V., *et al*. "Relation between Aluminum Concentrations in Drinking Water and Alzheimer's Disease: An 8-Year Follow-up Study". *American Journal of Epidemiology* 152.1 (2000): 59-66.

5. Shore D and Wyatt R. "Aluminum and Alzheimer's Disease". *Journal of Nervous and Mental Disease* 171.9 (1983): 553-558.

6. Kawahara M and Kato-Negishi M. "Link between Aluminum and the Pathogenesis of Alzheimer's Disease: The Integration of the Aluminum and Amyloid Cascade Hypotheses". *International Journal of Alzheimer's Disease* (2011): 312-320.

7. Tomljenovic L. "Aluminum and Alzheimer's Disease: After a Century of Controversy, Is There a Plausible Link?" *Journal of Alzheimer's Disease* 23.4 (2011): 567-598.

8. Huat TJ., *et al*. "Metal Toxicity Links to Alzheimer's Disease and Neuroinflammation". *Journal of Molecular Biology* 431.9 (2019): 1843-1868.

9. Torrealba E., *et al*. "In-Out-Test: A New Paradigm for Sorting the Wheat from the Chaff in Prodromal Alzheimer's Disease". *Journal of Alzheimer's Disease* 67.1 (2019): 265-277.

10. Ohkawa H., *et al*. "Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction". *Analytical Biochemistry* (1979): 351-358.

11. Bondy SC. "Prolonged Exposure to Low Levels of Aluminum Leads to Changes Associated with Brain Aging and Neurodegeneration". *Toxicology* 315.1 (2014): 1-7.

12. Akinrinade ID., *et al*. "Interplay of Glia Activation and Oxidative Stress Formation in Fluoride and Aluminium Exposure". *Pathophysiology* 22.1 (2015): 39-48.